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Original Article

BIOINFORMATIC CHARACTERIZATION OF SOD UNDER UV IN FENUGREEK (FAMILY: FABACEAE)

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ABSTRACT

Objective: To perform *In silico* analysis of superoxide dismutase (SOD) of the Fabaceae family along with the assessment of its physiological significance under UV irradiation in fenugreek belongs to the same family.

Methods: *In silico* study was carried out for the superoxide dismutase of Fabaceae and isozyme expression in the fenugreek was measured subjected to UV exposure during the post-germination phase.

Results: The expression of the enzyme was found constitutive in both the treated and control sets. On gel analysis exhibited a significant enhancement in the expression of enzymes under UV exposure especially at 365 nm. Computational analysis of SOD of Fabaceae revealed that the physicochemical properties and secondary structural features of the isoforms differ to some extent. It was also found that the motif sharing pattern and the conserved domain were different for each isoform, which may be attributed to the difference in cofactor involved.

Conclusion: The results suggest that the elevation of SOD activity might be responsible towards overcoming the stress imposed by UV irradiation. Thus, the role of SOD may be beneficial for oxidative stress management in plants during germination phases.

Keywords: *In-silico* Analysis, Superoxide dismutase, *Trigonella foenum-graecum*, UV irradiation

INTRODUCTION

Fenugreek, *Trigonella foenum-graecum* L., belonging to family Fabaceae, is used as a traditional medicine since ancient times. It is commonly known as seed spice used to improve flavor, colour and taste of food. Fenugreek has been known to possess potent antioxidant; anti-diabetic, anti-cataract, anti-hepatotoxic and immune-modulatory activities [1].

Ultraviolet (UV) radiation despite of its deleterious impact on plant system, including DNA damage, mechanical damage to photosynthetic apparatus and degradation of protein synthesis has been positively implemented in various researchers with sublethal dosage. Several reports have suggested that UV radiations have been successfully used in the extension of shelf life of fruits and vegetables. The application of UV treatment in several plant systems has resulted in the enhancement of bioactive phytochemical content and quality composition [2]. Reports have revealed the mitigation of stress by UV irradiation such as salinity stress in lettuce plants [3] and alleviation of injury due to chilling stress in pepper [4].

Superoxide dismutases (SODs) are enzymes that catalyze the reaction involved in the conversion of superoxide radical ($O_2^{\bullet-}$) to oxygen (O_2) and hydrogen peroxide and act as a first defense mechanism. Based on the metal co-factor acquired by them, SOD can be classified into four categories: copper-zinc SOD (CuZnSOD) which possesses copper and zinc together as redox active metal, iron SOD (FeSOD) which have iron, manganese SOD (MnSOD) which have manganese as redox active metal, and nickel SOD (NiSOD) having nickel metal co-factor. All these SOD types are present in prokaryotes while in eukaryotes FeSOD is present in chloroplasts, MnSOD is the SOD typically found in mitochondria and also in peroxisomes, and CuZnSOD is usually the most abundant among all and is present in the chloroplast, cytosol and also in the extracellular space [5]. Therefore, it can be considered that SOD is fairly ubiquitous in aerobic organisms. Several studies have suggested the active involvement of SOD in oxidative stress management during a wide spectrum of biotic as well as abiotic stresses. Considering the above fact, the present study is aimed to evaluate the SOD enzyme expression using biochemical as well as computational analysis under the influence of UV irradiation.

MATERIALS AND METHODS

Chemicals

Sodium hypochlorite, potassium hydroxide, potassium dihydrogen phosphate, polyvinylpyrrolidone, EDTA, triton, acrylamide, N,N'-Methylene-*bis(acrylamide)*, Tris-HCl, HCl, nitroblue tetrazolium, riboflavin and TEMED were either purchased from Sigma Chemicals (USA) or Merck (India) of analytical grade.

Elicitation process

The seeds of *Trigonella foenum-graecum* (Methi-P. E. B) were purchased from National Seeds Corporation Limited, Beej Bhavan, Pusa Complex, New Delhi and was further identified and authenticated by Professor A. P. Das, Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal. Dry seeds were sterilized by soaking in a solution of 1% (v/v) sodium hypochlorite. After sterilization, the seeds were exposed to UV rays of different wavelength i.e., 254 nm for 10 min, 365 nm for 10 min and both 254 nm and 365 nm in combination (COMB) for 5 min each. The dosage applied were equivalent to 1.27 kJ m^{-2}, 1.94 kJ m^{-2} and 1.61 kJ m^{-2} for 254 nm, 365 nm and combination respectively. The untreated seeds were used as a control set. For UV exposure, the seeds were placed under a 6 watt UV germicidal lamp (VL-6. LC 254 nm, Vilber Lourmat, France) at a distance of 5 cm from seeds. The intensity of irradiation was determined by a photo-radiometer (HD2102.2; Delta OHM, Padova, Italy).

Detection of Isozyme pattern by gel electrophoresis

The tissues were homogenized with ice-cold 50 mM potassium phosphate buffer (pH 7.0) including 1% polyvinyl pyrrolidone, 2 mM EDTA and 0.1% triton [6]. The homogenate was centrifuged at 10,000g for 20 min at 4˚C and the obtained supernatant was used for further detection of isozymes. The isoforms of superoxide dismutase (SOD; EC 1.15.1.1) were separated on discontinuous polyacrylamide gels (composed of 5% stacking gel and 10% resolving gel) under non-denaturing conditions. Proteins were electrophoresed at 4˚C and 80V in the stacking gel followed by 120V in the resolving gel.

The isozyme pattern of superoxide dismutase activity was detected on gel as described by Pereira *et al.* [6]. The gels were rinsed in distilled water and incubated in an assay mixture containing 0.05M potassium phosphate buffer (pH 7.8), EDTA (1 mM), nitroblue tetrazolium (0.1 mM), riboflavin (0.05 mM) and 0.3% N,N',N',N'-tetramethyl-ethylenediamine (TEMED) in the dark condition for 30 min at room temperature. After incubation, the gels were again rinsed with distilled water and then illuminated on a light box until the colourless bands of SOD appeared against a purple-stained gel background. The gel was scanned using GS-800 densitometer, BIORAD made. The relative density of the SOD isoforms was detected by Image lab (Version 5.1, BIORAD) software.

In silico analysis

The FASTA format of protein sequences of SOD of various plants of Fabaceae family was selected from the protein database of NCBI (National Centre for Biotechnology Information, (http://www.ncbi.nlm.nih.gov/protein/) as no sequence of SOD of fenugreek [*Trigonella foenum-graecum*; Family: Fabaceae] was recorded in the same database. The secondary structural properties of SOD were computed using SOPMA (Self Optimized Prediction Method with Alignment), (http://npsapbil.ibcp.fr/cgibinnpsa_ automat.pl?page=/NPSA/npsa_ sopma.html) tool of NPS (Network Protein Sequence Analysis). PDB structure analysis was performed with RCSB Protein Data Bank (http://www.rcsb.org/pdb/). The Procheck statistics were analyzed from the website http://www.ebi.ac.uk/pdbsum. CDD tool (Conserved Domain Database) from NCBI (http://www.ncbi. nlm.nih.gov/cdd) was implemented for domain and family analysis of amino acid sequence and Motif analysis was performed by MEME (meme-suite. org) and functional motif analysis of SOD by Expasy PROSITE (http://prosite.expasy.org/). The Expasy Protparam tool (http://web.expasy.org/protparam/) was applied for computing the physiochemical characterization of SOD. Phylogeny analysis of SOD of a different plant of Fabaceae family was aligned by ClustalW tool (http://www.ebi.ac.uk/ Tools/ msa/clustalw2/), and a phylogenetic tree was constructed by MEGA version 6.06.

RESULTS AND DISCUSSION

On gel analysis

The on gel antioxidant activity of superoxide dismutase was determined by native polyacrylamide gels. On determining SOD activity, five isoforms of SOD was detected in the fenugreek sprouts (fig. 1A). The patterns of the isozymes were different for the control and the UV treated seedlings. The seedlings treated with UV 254 nm did not show a significant change in the activity of the isozymes except for isozyme-II. However, UV 365 nm and combination (254 nm+365 nm) exhibited a remarkable increase in the intensity of three isoforms-I, II and III, also a slight increase in the isoforms-IV and V thus indicating an increase in the SOD activity by UV irradiation. The relative density of each SOD isoforms is represented in the fig. 1B where the increase in the SOD activity was evident.

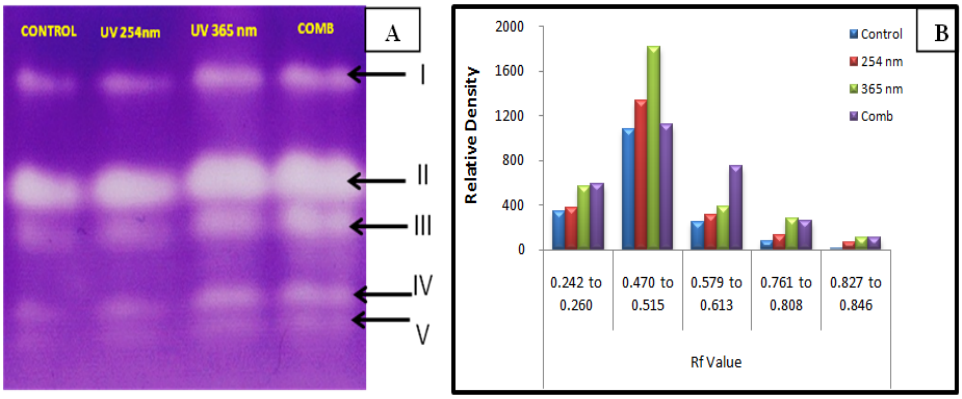

Fig. 1: Effect of UV exposure on the SOD activity of the fenugreek seedlings. A) On gel visualisation of SOD activity of treated and untreated seedlings, the arrows show the band corresponding to isoforms. B) The relative density of the SOD isoforms as detected by Image lab (Version 5.1, BIORAD) software
Rf value: Relative front; Comb: 254 nm and 365 nm in combination

Plants have evolved various protective mechanisms for minimizing such deleterious effects of different free radicals. Among the defense strategies, both enzymatic and non-enzymatic mechanisms are involved. The enzymatic defense comprises of the efficient antioxidant enzymes such as catalase, peroxidase, superoxide dismutase, polyphenol oxidase, etc [7]. In the present study, the native on gel analysis was performed for superoxide dismutase. Studying ROS metabolism related to UV exposure includes investigation of enzymatic components of the antioxidant system [8]. It has also been reported that UV irradiation leads to enhancement in the activity of the antioxidant system, as a defense mechanism [7]. Oxidative stress is accompanied by the excessive production of free radicals, among which superoxide radical is considered one of the most reactive, which is efficiently detoxified by superoxide dismutase in the cellular system [9].

It was observed that the intensity of the SOD isozymes was found to increase in the lane loaded with protein extracted from seedlings subjected to UV irradiation, which indicates an increase in the antioxidant activity of SOD enzyme. Also, the increase in the SOD activity might be attributed to the production of superoxide radical due to UV irradiation as earlier suggested by Dai *et al.* [10] in leaves of *Oryza sativa*. An enhancement in the activity of these antioxidant enzymes might be an acclimatization mechanism by the fenugreek seedlings to overcome oxidative stress imposed by UV irradiation. A wide variation in antioxidant system responses has been reported under UV exposure, depending on the plant species and intensity of radiation [10].

In silico study

Secondary structure properties, PDB Structure, Procheck statistics, Motif and Physicochemical characterization, for all SODs of the members of Fabaceae, were performed by implementing various computational tools. SOPMA analysis for SOD in the selected plants of the Fabaceae showed that alpha helix occupied the largest part of the protein followed by the random coil, extended strand and beta turns in case of FeSOD and MnSOD except for *Medicago truncatula*, where random coil occupied the larger part than the alpha helix. Interestingly in CuZnSOD, the trend was simply different, random coil occupied the largest part of the protein followed by, alpha helix, extended strand and beta turn and also in some plants the alpha helix were the least (table 1). It is suggested that high value for random coil bears important significance in the study of protein tertiary structure and related functions. Functional analysis of these proteins includes identification of important motifs (table 2).

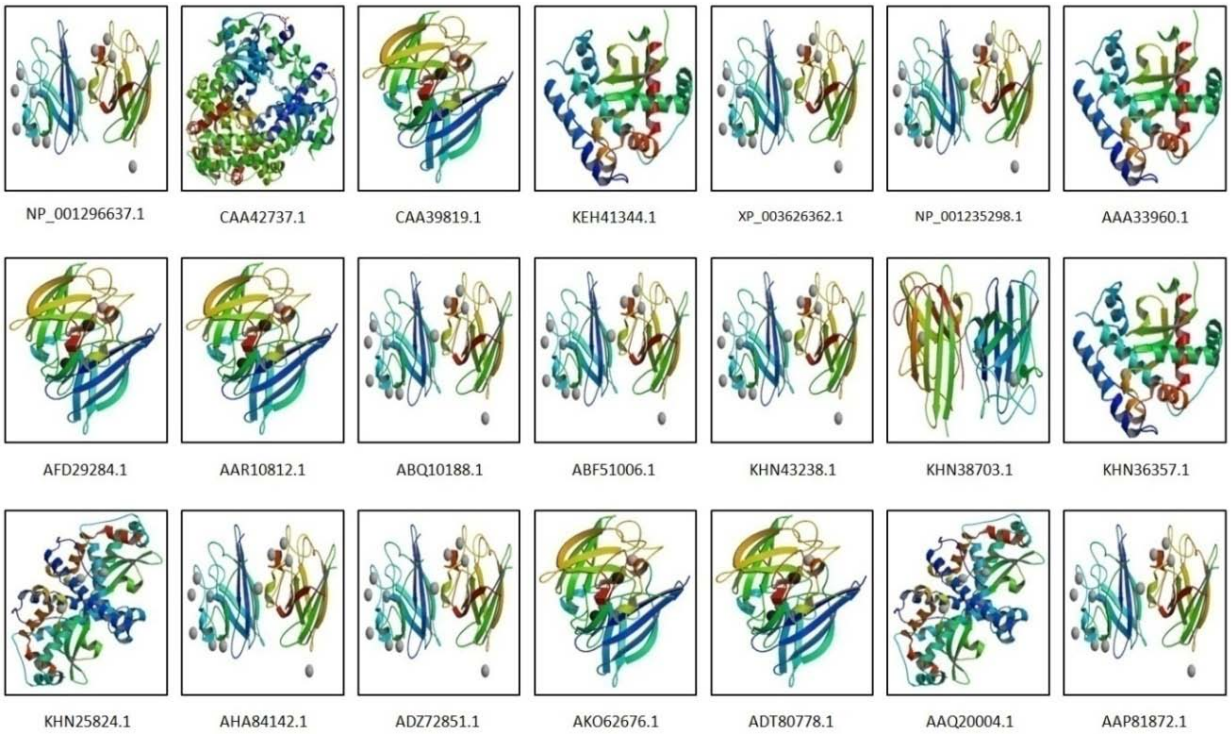

Fig. 2: Protein data bank structure of different SOD types in selected plants of fabaceae

Table 1: Secondary structure prediction of different SOD types in selected Fabaceae plants by SOPMA

S. No.	Plant SOD	α Helix	3_{10} Helix	Pi Helix	β Bridge	Extended Strand	β Turn	Bend Region	Random Coil	Ambiguous States	Other States
1	Cu/Zn *Cicer arietinum*	9	0	0	0	50	13	0	80	0	0
2	Cu/Zn *Pisum sativum*	38	0	0	0	67	20	0	77	0	0
3	Cu/Zn *Medicago truncatula*	6	0	0	0	52	13	0	81	0	0
4	Cu/Zn *Glycine max*	9	0	0	0	53	16	0	74	0	0
5	Cu/Zn *Vicia faba*	33	0	0	0	67	18	0	84	0	0
6	Cu/Zn *Trifolium pratense*	38	0	0	0	66	24	0	74	0	0
7	Cu/Zn *Caragana jubata*	10	0	0	0	51	14	0	77	0	0
8	Cu/Zn *Arachis hypogaea*	10	0	0	0	50	15	0	77	0	0
9	Cu/Zn *Glycine soja*	9	0	0	0	53	16	0	74	0	0
10	Cu/Zn [chloroplastic] *Glycine soja*	54	0	0	0	51	21	0	78	0	0
11	Cu/Zn *Phaseolus vulgaris*	7	0	0	0	53	15	0	77	0	0
12	Cu/Zn *Vigna radiata*	8	0	0	0	53	13	0	78	0	0
13	Cu/Zn *Stylosanthes guianensis*	44	0	0	0	59	19	0	85	0	0
14	Cu/Zn *Galega orientalis*	49	0	0	0	56	20	0	74	0	0
15	Cu/Zn *Lotus japonicus*	13	0	0	0	49	18	0	72	0	0
16	Mn *Pisum sativum*	92	0	0	0	50	29	0	69	0	0
17	Mn [mitochondrial] *Glycine soja*	86	0	0	0	58	29	0	68	0	0
18	Mn *Lotus japonicus*	88	0	0	0	50	25	0	83	0	0
19	Fe *Medicago truncatula*	102	0	0	0	64	19	0	126	0	0
20	Fe *Glycine max*	99	0	0	0	47	20	0	82	0	0
21	Fe [chloroplastic] *Glycine soja*	117	0	0	0	44	22	0	79	0	0

Table 2: Motif prediction of different SOD types in selected Fabaceae plants

S. No.	Plant SOD	Motif ID	Start	End	Metal signature 1	Motif ID	Start	End	Metal signature 2
1	Cu/Zn *Cicer arietinum*	PS00087	43	53	GFHIHAlGDtT	PS00332	137	148	GNAGgRvACgiI
2	Cu/Zn *Pisum sativum*	PS00087	92	102	GFHLHEyGDtT	PS00332	186	197	GNAGgRlACgvV
3	Cu/Zn *Medicago truncatula*	PS00087	43	53	GFHIHAlGDtT	PS00332	137	148	GNAGgRvACgiI
4	Cu/Zn *Glycine max*	PS00087	43	53	GFHVHAlGDtT	PS00332	137	148	GNAGgRvACgiI
5	Cu/Zn *Vicia faba*	PS00087	92	102	GFHLHEyGDtT	PS00332	186	197	GNAGgRlACgvV
6	Cu/Zn *Trifolium pratense*	PS00087	92	102	GFHLHEyGDtT	PS00332	186	197	GNAGgRlACgvV
7	Cu/Zn *Caragana jubata*	PS00087	43	53	GFHVHAlGDtT	PS00332	137	148	GNAGgRvACgiI
8	Cu/Zn *Arachis hypogaea*	PS00087	43	53	GFHVHAlGDtT	PS00332	137	148	GNAGgRvACgiI
9	Cu/Zn *Glycine soja*	PS00087	43	53	GFHVHAlGDtT	PS00332	137	148	GNAGgRvACgiI
10	Cu/Zn [chloroplastic]*Glycine soja*	PS00087	94	104	GFHLHEyGDtT	PS00332	188	199	GNAGgRlACgvV
11	Cu/Zn *Phaseolus vulgaris*	PS00087	43	53	GFHVHAlGDtT	PS00332	137	148	GNAGgRvACgiI
12	Cu/Zn, *Vigna radiata*	PS00087	43	53	GFHVHAlGDtT	PS00332	137	148	GNAGgRvACgiI
13	Cu/Zn *Stylosanthes guianensis*	PS00087	97	107	GFHLHEyGDtT	PS00332	191	202	GNAGgRlACgvV
14	Cu/Zn *Galega orientalis*	PS00087	89	99	GFHLHEyGDtT	PS00332	183	194	GNAGgRlACgvV
15	Cu/Zn *Lotus japonicus*	PS00087	43	53	GFHVHAlGDtT	PS00332	137	148	GNAGgRvACgiI
16	Mn *Pisum sativum*	PS00088	201	208	DvWEHAYY				
17	Mn [mitochondrial] *Glycine soja*	PS00088	202	209	DvWEHAYY				
18	Mn *Lotus japonicus*	PS00088	206	213	DvWEHAYY				
19	Fe *Medicago truncatula*	PS00088	233	240	DvWEHAYY				
20	Fe *Glycine max*	PS00088	203	210	DvWEHAYY				
21	Fe [chloroplastic] *Glycine soja*	PS00088	210	217	DlWEHAYY				

During motif prediction analysis two important motifs were found in CuZnSOD (PS00087 and PS00332) and single motif (PS00088) was present in both FeSOD and MnSOD. PS00087 occurred at the starting sequence, i.e. from 43-53 as well as a middle sequence like 94-104 whereas PS00332 and PS00088 occurred at the end sequence like 186-197 and 210-217 respectively. These motifs were found to be 8 to 12 amino acids in length arise because specific residues and regions are important for the biological functioning of a group of proteins, which are known to be conserved in structures and sequences during evolution. The ExPASy ProtParam tool was implemented for evaluating the amino acid composition (%) under four categories, namely; helix breaker which includes glycine and proline,-OH group-containing amino acids, which include serine, threonine and tyrosine, an amino acid with sulfhydryl group which is cysteine and the fourth category was amide group including asparagine and glutamine. It was observed that the percentage of helix breaker was higher than the amino acid with-OH group for most of the CuZnSOD whereas in case of FeSOD and MnSOD, the percentage of the amino acid with-OH group was higher than helix breaker. For sulfhydryl group the CuZnSOD (1-1.3%) a contained comparatively higher amount than FeSOD and MnSOD (0-0.4%) **(**fig. 3**)**. The total number of positively (Arg+Lys) and negatively (Asp+Glu) charged residues of SOD members were also evaluated (table 3). The result indicated that both positively and negatively charged nature of SOD varies with their isoelectric point. It was observed that all the CuZnSOD of analyzed plants showed a comparatively high negative charge, but MnSOD exhibited higher positively charged amino acids. Such difference might be attributed to their isoelectric point (pI) as it was evident from table 3 that those having higher negative charge had their pI in the acidic range and those with positive charge ≥ negative charge had their pI above 7.00.

For the separation of the isoforms of the SOD on a polyacrylamide gel the isoelectric point must have a vital role [11]. Interestingly, a similarity in the physicochemical properties such as sulphur containing amino acids (fig. 3) and the isoelectric point (pI) (table 3) was observed between CuZnSOD and FeSOD which has been also reported in previous studies [12]. A significant difference in the Extinction coefficient (ε) for SOD isoforms was also observed; the ε of MnSOD and FeSOD (53400-62005) was much higher than that of CuZnSOD (125-1615).

The ε value is attributed to higher concentration of amino aromatic acids such as lysine, tryptophan, and tyrosine, and interestingly, it was found that the MnSOD and FeSOD had a higher concentration of these aromatic amino acids than CuZnSOD. The value of ε has been considered to be very important for the determination of protein concentration. The degree of stability of proteins can be determined by the value of the instability index (II), the higher II value indicates higher instability or lower stability but, the lower II value indicates that the protein is highly stable. It was observed that almost all the SOD exhibited Instability index less than 40 except for that of FeSOD of *Medicago truncatula* was above 40 (42.02) thus indicating the protein is unstable. The higher aliphatic index signifies higher concentration of alanine, valine, leucine and isoleucine occupying the relatively large volume of protein**.** In the present study high aliphatic index was exhibited by all the SOD isoforms of analysed Fabaceae plants. GRAVY (Grand Average of Hydropathy) signifies the degree of solubility of protein depending upon their hydrophobicity or hydrophilic nature. On analysis of GRAVY index (table 3) it was observed that most of the SODs were hydrophilic with negative value (-0.006 to -0.690) and few were found to be hydrophobic with positive value (0.004 to 0.063).

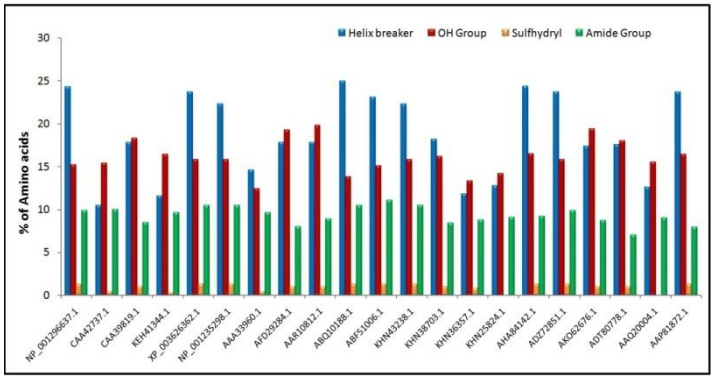

Fig. 3: Functional representation of amino acids of SOD in selected plants of fabaceae

Table 3: Parameters computed using ExPASy ProtParam tool of different SOD types in selected Fabaceae plants

S. No.	Plant SOD	Accession no.	No. of amino acids	MW	pI	-R	+R	EC	II	AI	GRAVY
1	Cu/Zn *Cicer arietinum*	NP_001296637.1	152	15221.7	5.44	15	8	125	13.53	80.79	-0.206
2	Cu/Zn *Pisum sativum*	CAA39819.1	202	20610.2	5.94	17	12	1615	20.19	94.55	0.044
3	Cu/Zn *Medicago truncatula*	XP_003626362.1	152	15226.7	5.45	15	8	125	6.01	76.32	-0.274
4	Cu/Zn *Glycine max*	NP_001235298.1	152	15193.6	5.27	15	7	125	10.62	78.22	-0.172
5	Cu/Zn *Vicia faba*	AFD29284.1	202	20629.1	5.79	18	12	1615	17.63	92.13	0.005
6	Cu/Zn *Trifolium pratense*	AAR10812.1	202	20658.1	5.79	17	11	1615	18.19	92.62	-0.006
7	Cu/Zn *Caragana jubata*	ABQ10188.1	152	15163.7	5.82	14	9	125	9.23	77.57	-0.241
8	Cu/Zn *Arachis hypogaea*	ABF51006.1	152	15197.6	5.46	15	8	125	14.07	76.91	-0.270
9	Cu/Zn *Glycine soja*	KHN43238.1	152	15193.6	5.27	15	7	125	10.62	78.22	-0.172
10	Cu/Zn [chloroplastic] *Glycine soja*	KHN38703.1	204	20900.5	6.03	18	14	1615	30.58	91.76	0.018
11	Cu/Zn *Phaseolus vulgaris*	AHA84142.1	152	15186.6	5.59	15	8	1615	11.24	72.43	-0.295
12	Cu/Zn *Vigna radiata*	ADZ72851.1	152	15255.7	5.59	15	8	1615	12.49	74.43	-0.278
13	Cu/Zn *Stylosanthes guianensis*	AKO62676.1	207	21107.7	5.78	17	11	1615	18.25	92.75	0.063
14	Cu/Zn *Galega orientalis*	ADT80778.1	199	20355.8	5.74	19	12	1615	19.31	94.07	0.023
15	Cu/Zn *Lotus japonicus*	AAP81872.1	152	15113.6	5.64	15	9	125	12.92	78.88	-0.170
16	Mn *Pisum sativum*	CAA42737.1	240	26637.3	7.16	24	24	57410	30.83	95.58	-0.254
17	Mn [mitochondrial] *Glycine soja*	KHN25824.1	241	26706.4	8.56	24	26	54890	27.84	95.15	-0.285
18	Mn *Lotus japonicus*	AAQ20004.1	246	26906.5	7.20	23	23	53400	32.87	91.26	-0.211
19	Fe *Medicago truncatula*	KEH41344.1	311	35332.3	5.45	46	35	57410	42.02	68.10	-0.690
20	Fe *Glycine max*	AAA33960.1	248	27841.6	5.60	30	25	57410	35.48	82.18	-0.312
21	Fe [chloroplastic] *Glycine soja*	KHN36357.1	262	30318.6	5.83	34	29	62005	39.51	85.27	-0.329

MW: Molecular weight; pI: Isoelectric point; -R: negatively charged residues; +R: positively charged residues; EC: Extinction Coefficient; II: Instability Index; AI: Aliphatic Index; GRAVY: Grand Average of Hydropathy

Domain and MEME analysis

Bioinformatic analysis revealed that the 3 types of SOD belong to three different domains having following CDD accession cd00305 (CuZnSOD), PLN02471 (MnSOD) and further it was observed that FeSOD belongs to domain PLN02685, PLN02184 and PLN02622 (fig. 4).

For motif searching Multiple Expectation-Maximization for Motif Elicitation (MEME) suite tool was implemented [11]. Thirty different motifs were detected and distributed by MEME software. The motifs were found to be shared in the different pattern by 3 types of SODs of analysed plants (fig. 5). Motifs 1, 2, 3 and 6 were found to be conserved in CuZnSOD as they are shared by all the CuZnSODs in the almost similar pattern. Motifs 4, 5 and 7 were found to be conserved in the FeSOD and MnSOD and further, it was observed that these two SODs have a different set of conserved motif individually, motifs 10, 13, 14 and 19 were found to be conserved in MnSOD and motifs 9, 15 and 28 was conserved in FeSOD (fig. 5).

Such difference in their motif distribution may be attributed to the type of metal cofactor harbored by them. Other motifs were also found to be limited in very few plants, and such Motifs may be the consequence of substitution or accumulation of mutation or rearrangements.

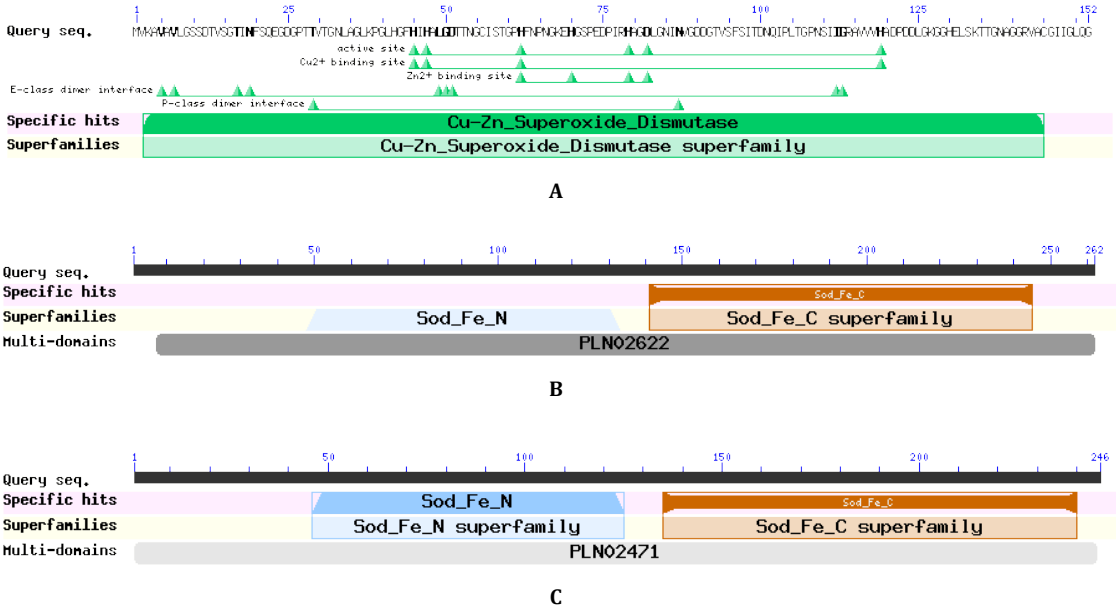

Fig. 4: Functional domain analysis of A) CuZnSOD B) MnSOD and C) FeSOD of selected fabaceae plants

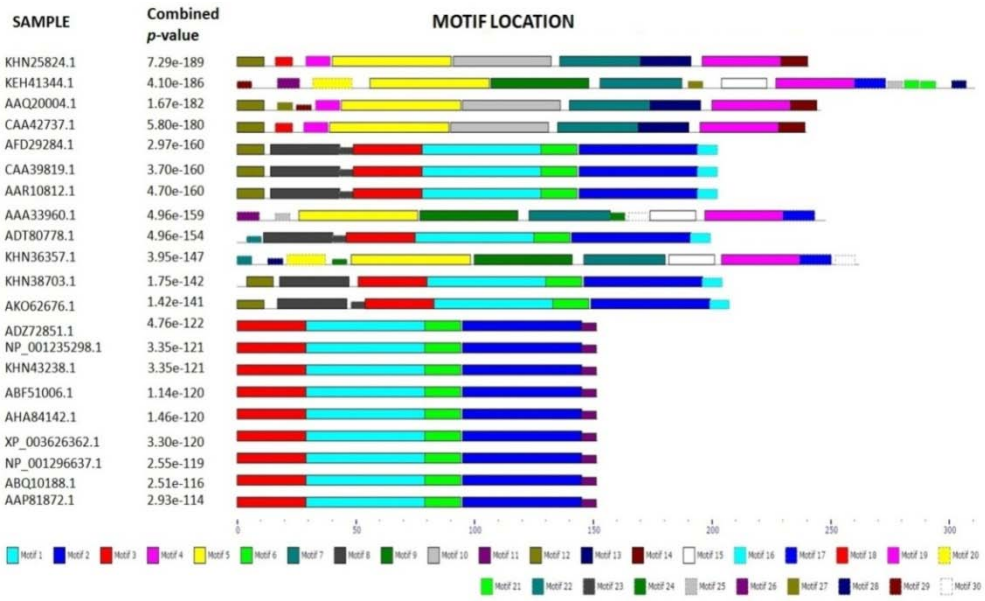

Fig. 5: Schematic diagram of motif distribution of different SOD types in selected fabaceae plants

Phylogenetic analysis

Phylogenetic analysis was performed using the distance-based neighbour-joining method. The pattern of the phylogenetic tree obtained was in agreement to the idea hypothesized by Martin and Fridovich [13] who earlier proposed that FeSODs are far antique and the CuZnSODs evolved independently of FeSODs and MnSODs Two main clusters were obtained from phylogenetic tree; in one clade all CuZnSOD were clustered together, whereas the other clade was split into two distinct groups; one having all MnSOD and the other belongs to FeSOD of selected Fabaceae plants suggesting that these two, FeSOD and MnSOD were originated from common ancestors. Similar observations were cited in earlier reports regarding the SODs of other plants [14,15]. Among CuZnSOD, *Cicer arietinum* was out grouped from other Fabaceae representatives (fig. 6). Interestingly the chloroplastic CuZnSOD of *Glycine soja* was incorporated in the same cluster of cytosolic CuZnSOD, indicating no major sequence changes during organelle-specific localization. Thus, the structural divergence of SOD isozyme in Fabaceae was associated with functional specialization depending on metal ions.

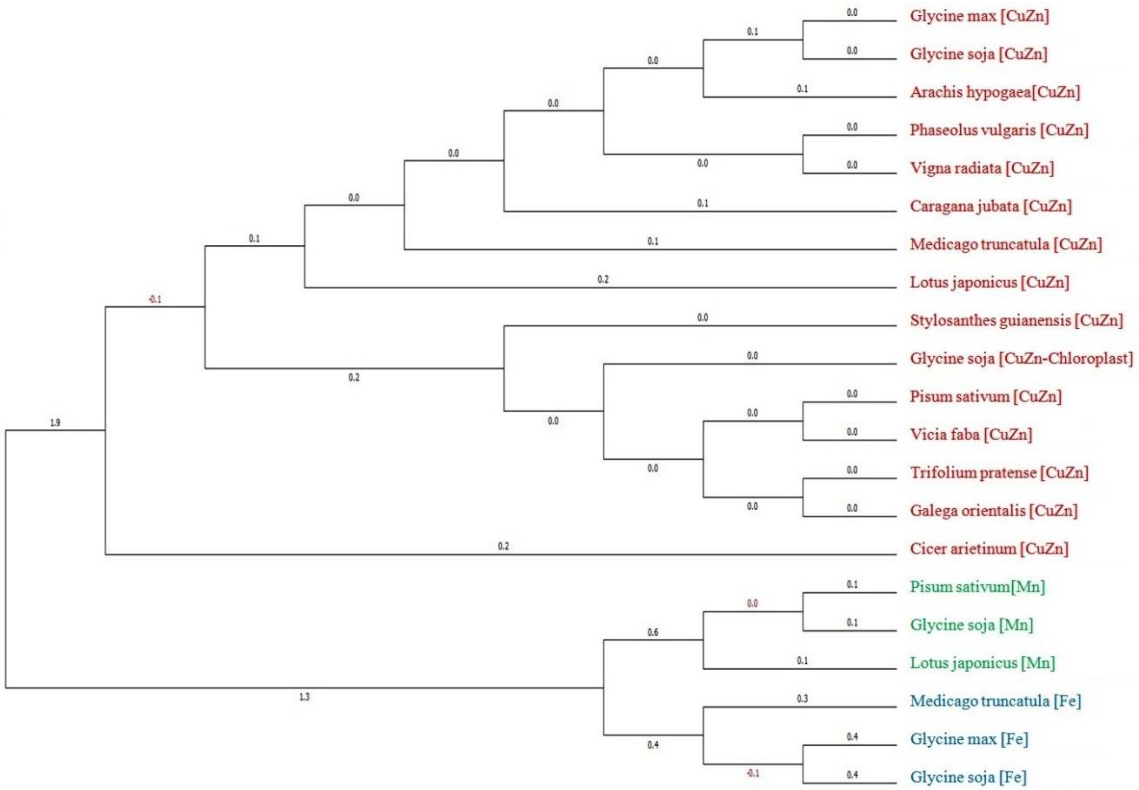

Fig. 6: Phylogenetic tree of different SOD types in selected fabaceae plants

CONCLUSION

When subjected to the UV exposure the SOD activity was found to be highly expressed than in control set. The SOD activity was found to be enhanced under UV irradiation at 365 nm. The various results obtained by the *in-silico* studies of the SOD enzyme will provide insight for all those researchers involved in understanding the functionality of SOD enzyme and the role of different metal cofactors associated with this antioxidant enzyme. Further, it can be explained that the UV irradiation technology might be implemented for the induction of the antioxidant enzyme system which possibly will be beneficial for oxidative stress management in plants during germination phases.

ABBREVIATION

SOD: Superoxide Dismutase, UV: Ultra Violet, CuZnSOD: Copper-Zinc SOD, FeSOD: Iron SOD, MnSOD: Manganese SOD, COMB: 254 nm and 365 nm in combination, EDTA: Ethylenediaminetetraacetic acid, TEMED: N, N,N′,N′-tetramethyl-ethylenediamine, MEME: Multiple Expectation-Maximization for Motif Elicitation

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CONFLICT OF INTERESTS

The authors have no competing interests to declare.

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